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(54) Title: IN OVO VACCINATION AGAINST NEWCASTLE DISEASE

(57) Abstract

The present invention is concerned with a vaccine for *in ovo* vaccination of poultry against Newcastle Disease Infections. This vaccine contains Newcastle Disease Viruses of the strain with the internal indication NDW, deposited at CNCM (Institut Pasteur) under number I–781.

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IN OVO VACCINATION AGAINST NEWCASTLE DISEASE

The present invention is concerned with a vaccine suitable for *in ovo* vaccination against Newcastle Disease infection, with the use of a Newcastle Disease Virus strain in the preparation of such a vaccine, as well as with the protection of poultry against Newcastle Disease infection by *in ovo* vaccination with a vaccine containing a Newcastle Disease virus train.

In ovo vaccination of virus-containing vaccines was extensively described by Sharma et al. (US Patent No 4458630). In particular it teaches that live Marek's disease virus can be injected into amniotic fluid within the egg, whereafter the embryo is infected and the vaccine virus replicates to a high titer which induces the formation of protective antibodies in the treated embryo. (Sharma; Avian Diseases 29, 1155, 1167-68 (1985).

In US Patent No 5427791 Ahmad et al. describe the embryonal vaccination against Newcastle Disease. Herein, in order to provide for a non-pathogenic attenuation of the live Newcastle Disease virus (strain NDV-B1), the viruses were modified through use of ethyl methane sulfonate (EMS).

A disadvantage of this type of modification is the fact that EMS is a mutagen and that the vaccine is suspected to act as a mutagen as well, which is undesirable for regular administration of the vaccine. On the other hand, untreated NDV-B1 cannot be applied for *in ovo* vaccination as almost all of the embryos will die upon injection of the eggs with this unmodified virus.

Furthermore, it has been found that the margin between minimum effective dose and the maximum dose for safety for these modified viruses is less than 10 (hence less than log1). For practical purposes and in view of the errors as a result of production and due to losses during storage, this margin is too small.

Surprisingly it has been found that a vaccine preparation containing Newcastle Disease viruses of the strain NDW is particularly suited for *in ovo* application. Hence the present invention is concerned with the use of Newcastle Disease virus of the strain NDW in the *in ovo* vaccination of poultry. As a further embodiment the invention is concerned with the use of Newcastle Disease virus of the strain NDW in the preparation of a vaccine suitable for *in ovo* administration poultry.

Samples of the Newcastle Disease virus strain NDW were deposited at CNCM of Institut Pasteur under No 781. See EP 351908.

Advantageously, the NDW strain is administered in an amount of between 10^{-1} and 10^{3} and more in particular in an amount between $10^{-0.7}$ and $10^{2.2}$ per egg.

For obtaining the best results in immunisation it was found that the NDW containing vaccine can be administered *in ovo* at between 17 and 19 days of incubation, preferably at 18 days of incubation.

Example 1

Preparation of NDW vaccine for *in ovo* administration

A Working Seed Virus stock was prepared from a Master Seed Virus (deposited at CNCM, Institut Pasteur under No I-781) by inoculation into the allantoic cavity of embryonated SPF chicken eggs.

In the same way the vaccine is produced by inoculation of Working Seed Virus into the allantoic cavity of embryonated SPF eggs. After incubation the allantoic fluid containing the vaccine virus is harvested. The allantoic fluid is diluted and frozen and stored at -50°C.

Before filling the allantoic fluid is thawed, further diluted until the required concentration of vaccine virus, mixed with stabiliser, filled into vials and freeze-dried.

Example 2

The Safety of the *in ovo* NDW vaccine in SPF eggs.

SPF eggs were vaccinated at 18 days of incubation in the amnion by the method described by Sharma and Burmester (Avian Diseases 26 (1), 134-149) with the vaccine described in Example 1.

Six groups of eggs were vaccinated according to the scheme outlined in the following Table.

Table 1: **Safety of *in ovo* vaccination of SPF eggs with NDW vaccine**

Group	Vaccine dose (in EID_{50})	Number of Eggs	Percentage hatch
1	$10^{2.2}$	25	76
2	$10^{1.2}$	25	84
3	$10^{0.2}$	25	84
4	$10^{-0.8}$	25	88
5	$10^{-1.8}$	25	92
6	controls	25	96

Conclusion: In ovo vaccination of SPF eggs at 18 days of embryonal development with NDV vaccine is safe with a maximum dose of between $10^{1.2}$ and $10^{2.2}$ EID_{50} per egg.

Example 3

The safety of the *in ovo* NDW vaccine in commercial broiler eggs with maternal antibodies.

Commercial broiler eggs having maternal antibodies were vaccinated at 18 days of incubation in the amnion by the method described by Sharma and Burmester (Avian Diseases 26 (1), 134-149) with the vaccine described in Example 1.

Eight groups of eggs were vaccinated according to the scheme outlined in the following Table.

Table 2: **Safety of *in ovo* vaccination of commercial broiler eggs with NDW vaccine**

Group	Vaccine dose (in EID_{50})	Number of Eggs	Percentage hatch
1	10^6	50	68
2	10^5	50	70
3	10^4	50	74
4	10^3	50	76
5	10^2	50	91
6	10^1	50	84
7	10^0	50	96
8	controls	50	88

Conclusion: *In ovo* vaccination of broiler eggs with maternal antibodies is safe (no effect on hatching) up to a dose of at least 10^2 EID_{50} per egg.

Example 4

Efficacy of *in ovo* vaccination of SPF eggs with NDV vaccine

The efficacy of NDV vaccine prepared according to Example 1 was examined in SPF eggs.

Parameters for the protection were the antibody response after vaccination (haemagglutination inhibition test = HI test) and percentage of mortality after challenge. The challenge virus was the strain Hertz 33/56 of Newcastle Disease Virus, with was administered to each of the chickens in an amount of $10^{5.0}$ EID_{50}.

Five groups of eggs were vaccinated according to the scheme outlined in the table below:

Table 3: **Efficacy of *in ovo* vaccination of SPF eggs.**

Group	Vaccine Dose (in EID_{50})	HI titer (in ^{2}log) at weeks		Percentage mortality after challenge at 4 weeks
		4	6	
1	$10^{1.7}$	4.6	nd	0
2	$10^{1.0}$	5.1	5.7	0
3	$10^{0.0}$	3.6	3.3	0
4	$10^{-0.7}$	3.3	3.2	11
5	control	1.0	1.0	100

Conclusion: In ovo vaccination of SPF eggs at 18 days of embryonal development with NDV vaccine is effective. A vaccine dose of about $10^{-0.7}$ per egg is the minimal effective dose for *in ovo* NDV vaccination.

Example 5

Efficacy of *in ovo* vaccination with NDV vaccine of commercial broiler eggs with maternal antibodies.

The efficacy of the NDV vaccine prepared according to Example 1 was examined in commercial broiler eggs with maternal antibodies (HI titer of 5.1 at one day of age).

Parameters for the protection were the antibody response after vaccination (HI test) and percentage of mortality after challenge. The challenge virus was the strain Hertz 33/56 of Newcastle Disease Virus, which was administered to each of the chickens in an amount of $10^{5.0}EID_{50}$.

Three groups of eggs were vaccinated according to the scheme outlined in the table below:

Table 4: **Efficacy of *in ovo* vaccination in commercial broiler eggs**

Group	Vaccine Dose (in EID_{50})	HI titer (in ^{2}log) at 3 weeks of age	Percentage of mortality after challenge at 3 weeks of age
1	10^2	3.9	0
2	10^{-1}	2.4	0
3	control	1.0	100

Conclusion: In ovo vaccination with NDV vaccine of commercial broiler eggs at 18 days of embryonal development is effective. This is not influenced by the presence of maternal antibodies.

CLAIMS:

1. A vaccine for *in ovo* vaccination of poultry against Newcastle Disease infections, characterised in that it comprises a virus having the characteristics of the strain NDW, an example of which is deposited at CNCM (Institut Pasteur) under number I-781.

2. A vaccine according to Claim 1 characterised in that the viruses of strain NDW are administered in an amount of between 10^{-1} and 10^{3} EID_{50} per egg.

3. A vaccine according to Claim 1 characterised in that the viruses of strain NDW are administered in an amount of between $10^{-0.7}$ and $10^{2.2}$ EID_{50} per egg.

4. A vaccine according to Claim 1 characterised in that the viruses of strain NDW are administered *in ovo* at between 17 and 19 days of incubation.

5. A vaccine according to Claim 1 characterised in that the viruses of strain NDW are administered *in ovo* at 18 days of incubation.

6. Use of a Newcastle Disease Virus strain NDW, deposited at CNCM (Institut Pasteur) under number I-781, in the preparation of a vaccine suitable for *in ovo* vaccination.

7. A use according to Claim 6, characterised in that the viruses of strain NDW are present in an amount of between 10^{-1} and 10^{3} per egg.

8. Use according to Claim 6, characterised in that the viruses of strain NDW are present in an amount of between $10^{-0.7}$ and $10^{2.2}$ per egg.

9. A method for preventing or treating Newcastle Disease infections in poultry comprising *in ovo* administration of a vaccine comprising a virus having the characteristics of the strain NDW an example of which is deposited at CNCM (Institut Pasteur) under number I-781.

INTERNATIONAL SEARCH REPORT

Int 'ional Application No
PCT/EP 97/07066

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 A61K39/17

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	EP 0 351 908 A (DUPHAR INT RES) 24 January 1990 ---	
A	DATABASE BIOSIS BIOSCIENCES INFORMATION SERVICE, PHILADELPHIA, PA, US Abstract no. 91:501771, VAN ECK J H H ET AL: "AN ULSTER 2C STRAIN-DERIVED NEWCASTLE DISEASE VACCINE VACCINAL REACTION IN COMPARISON WITH OTHER LENTOGENIC NEWCASTLE DISEASE VACCINES." XP002031012 see abstract & AVIAN PATHOL 20 (3). 1991. 497-508. CODEN: AVPADN ISSN: 0307-9457, --- -/--	

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

* Special categories of cited documents :

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Date of the actual completion of the international search	Date of mailing of the international search report
8 May 1998	20. 05. 1998

Name and mailing address of the ISA	Authorized officer
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INTERNATIONAL SEARCH REPORT

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C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	DATABASE BIOSIS BIOSCIENCES INFORMATION SERVICE, PHILADELPHIA, PA, US Abstract no. 91:501770, VAN ECK J H H ET AL: "AN ULSTER 2C STRAIN-DERIVED NEWCASTLE DISEASE VACCINE EFFICACY AND EXCRETION IN MATERNALLY IMMUNE CHICKENS." XP002031013 see abstract & AVIAN PATHOL 20 (3). 1991. 481-496. CODEN: AVPADN ISSN: 0307-9457, ---	
A	US 5 427 791 A (AHMAD JAMIL ET AL) 27 June 1995 cited in the application -----	

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INTERNATIONAL SEARCH REPORT

International application No.
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Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

Although claim 9 is directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.

2. [ ] Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

3. [ ] Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. [ ] As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. [ ] As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest.

[ ] No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

Information on patent family members

Int. ational Application No
PCT/EP 97/07066

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
EP 0351908 A	24-01-1990	AT 112490 T	15-10-1994
		DE 68918649 D	10-11-1994
		DE 68918649 T	23-03-1995
		DK 347189 A	19-01-1990
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		JP 2655726 B	24-09-1997
		US 5149530 A	22-09-1992
US 5427791 A	27-06-1995	NONE	